

## SEED PRODUCTION IN THE VIRUS INDICATOR PLANT SCOPOLIA SINENSIS

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### Abstract

A simple and effective means of producing seed of *Scopolia sinensis* Hemsl., a local lesion host of potato spindle tuber metavirus, is described. Pollinations made the day before or the day of flower opening resulted in good fruit and seed set, while pollinations made at the time of anthesis resulted in poor fruit set and almost no seed set. Pollen viability declined rapidly with storage. Freshly harvested seed germinated well. No natural seed dormancy was observed.

### Introduction

Previous reports (4,5) from this laboratory have shown that several species of *Scopolia* are indicator plants for potato spindle tuber metavirus<sup>2</sup> (PSTM). *Scopolia sinensis* Hemsl. was shown to develop local lesions with both mild and severe strains of PSTM (5) and appeared to be a suitable host for quantitative assay during purification studies (6).

The need for a reliable means of seed production from *S. sinensis* has been made evident by the demand for this plant as an indicator for PSTM. Workers desiring to employ this plant have found difficulty in propagating it vegetatively and have often expressed a desire for seed.

In our work, a collection of *S. sinensis* seed obtained from the Netherlands was the only seed which was found to be suitable for the indexing of PSTM. Seed of this particular lot was not available in sufficient quantities to permit continuous use of seedlings; therefore, the plants had to be multiplied by cuttings. Attempts to produce seed in the greenhouse failed and only a few seeds were obtained from plants grown in the field.

A survey of literature revealed that *Scopolia* species typically have low fertility because of several inherent abnormalities in the male and female gametophytes (1,2) or

because of an early shedding of flowers (3). Modification of environmental conditions (3) or use of growth stimulants (2,3) failed to increase flower retention and thus seed production has been erratic and unpredictable.

Here we report a simple procedure which lends itself readily to the production of seed in the greenhouse.

### Materials and methods

Vigorous plants of *S. sinensis* which had previously been multiplied vegetatively were selected for use in seed production. They were grown in a greenhouse during the winter and were subjected to an 18-hr day with supplemental fluorescent lighting of 600-700 ft-c. Temperatures were maintained at 21-24°C. These conditions promoted flower initiation.

Pollination was done at the time of anthesis or 1 to 3 days prior to anthesis. Flowers open approximately 1 day before anthesis. When buds were used, the upper portion of the corolla was removed to expose the stigma. No emasculation was done. Pollination was accomplished by removing a dehiscent anther from a flower of the same or preferably another plant and rubbing it repeatedly across the stigmatic surface until the stigma was well covered with pollen. Pollen was applied only once. Each pollinated flower was tagged and dated.

The viability of stored pollen was investigated. Dehiscent anthers were collected in small gelatin capsules and placed in sealed containers with anhydrous calcium chloride as a drying agent. These containers were placed in a refrigerator (4°C). Pollen viability was judged by stainability with acetocarmine and by fruit set.

Fruits were harvested 6-8 weeks after pollination. A sample of seed was used for germination tests immediately after harvest.

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<sup>2</sup> In view of the low-molecular weight nature of the spindle tuber agent and its other properties (reference 6), Singh and Clark have proposed the term "metavirus" to describe the infectious agent of spindle tuber disease. The details of the proposal will appear elsewhere.

## Results

Table 1 summarizes the data collected on fruit and seed set in flowers pollinated before and at anthesis. Fruit set was most successful in flowers pollinated the day before or the day of opening (pre-anthesis). Fruits from pre-anthesis pollinations were larger and contained more seeds than those from anthesis pollinations. The flowers pollinated at anthesis set only a few fruits, most of which were seedless.

It was noted from the examination of ovaries from unsuccessful pollinations that often the only ovules found to be developing from anthesis pollinations were located in the upper portion of the ovary, while those developing from pre-anthesis pollinations occurred over the entire placental surface. In an attempt to enhance the success of fertilization, styles of anthesis stage flowers were cut in half and pollinated on the cut surface; however, developing ovules were still found primarily in the upper portion of the ovaries. The "at style" approach was found to be ineffective.

Stored pollen lost its viability very rapidly. Stainability fell from more than 90% on the first day to about 70% by the third day of storage. Pollen stored for several weeks was still capable of inducing fruit set; however, to ensure success, use of pollen which has been stored more than a few days is not recommended.

Seed requires 6 to 12 days to germinate. About 75% of seeds planted immediately after harvest germinated. Seed of this plant does not seem to exhibit dormancy.

The resultant seedlings produced numerous local lesions in response to inoculation with PSTM, thus retaining the characteristic of the parental line.

## Discussion

Why seed production should fail when flowers are pollinated at the time of anthesis is an open question. When a flower first opens, the anthers lie below the stigma but by the time of anthesis, the stamens have elongated, placing the anthers above the

stigma. The time between the opening of the flowers and anthesis is about one day. This suggests that pre-anthesis pollination might be the natural mode of pollination in the wild where visitation by insects would be the most likely mechanism of pollination. The lack of successful seed production at anthesis may simply be a reflection of an effective mechanism which promotes cross pollination by taking advantage of a time differential between female receptivity and anthesis.

The method herein described to obtain seed set from *S. sinensis* is simple and requires no unusual techniques or facilities. Its implementation should make seed production possible wherever this plant will flower.

## Literature cited

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Table 1. Fruit and seed set in *Scopolia sinensis* plants pollinated at different stages of flower development<sup>1</sup>

Flower stage when pollinated	No. of flowers pollinated	No. of fruit	No. of fruit harvested	No. of seeds	No. of seeds/fruit (avg)	(range)
Pre-anthesis <sup>2</sup>	132	59	19	548	29	0-95
Anthesis	60	7	6	2	0.3	0- 2

<sup>1</sup> Data collected during January and February 1972.

<sup>2</sup> 1-3 days before anthesis.